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No supplementary notes

14. ABSTRACT:

Lung cancer is a deadly malignancy. The most commonly mutated oncogene we know is KRAS. We do not have a drug to inhibit KRAS, and medicinal chemistry approaches have exhausted leads. We need a new approach to find new ways to inhibit this oncogene.
Objective: To identify cellular cofactors required for KRAS ^{G12D}-driven NSCLC.

Specific Aim 1. To identify gene products specifically essential for KRAS-driven NSCLC, we will perform a shRNA screen of thousands of mouse genes, looking for essentiality in multiple independent cell lines derived from two NSCLC GEMMs: one RAF-dependent and one RAS-dependent. **This Aim is underway and has verified that KRAS is indeed essential in the KRAS mutant mouse cell lines.**

Specific Aim 2. To validate our findings in human NSCLC we will test a panel of human NSCLC cell lines with known dependence on RAS for their dependence on the elements (hits) identified in Aim 1.

Study Design: *In vitro* shRNA screen in Aim 1. Individual shRNA validation into human lines in Aim 2.

15. SUBJECT TERMS Nonsmall cell lung cancer, shRNA screening

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The text of the report must include all sections addressed in the table of contents to include:

INTRODUCTION: As targeted therapies improve the lives of many with certain types of lung cancer, KRAS-mutant non-small cell lung cancer (NSCLC) stands out as a deadly and poorly treated exception. We have no effective therapies for these patients. This project innovates and reinvigorates the search for direct ways to inhibit KRAS mutant NSCLC, a challenge we must meet to make progress in this clinically challenging NSCLC subset.

Mutant KRAS, like ALK or EGFR, is a *bone fide* NSCLC driver. Unlike ALK or EGFR however, KRAS is not a kinase. The traditional chemical screening approach to drug development is poorly suited for non-kinases in general and for the RAS inhibitor problem in particular. Chemical screening underperforms because *inhibiting* RAS would paradoxically require a compound to *reactivate* the RAS GTPase or act though some other novel mechanism. As such, drug companies' target protein-directed screening techniques have and will continue to fail in this endeavor. Innovative screening platforms and/or methods are desperately needed.
Hypothesis: KRAS does not signal alone. A genetically encoded cofactor (e.g. structural protein or noncoding RNA or metabolic regulator) is required for mutant KRAS to drive NSCLC. Since autochthonous $KRAS^{G12D}$ driven GEMMs develop NSCLC, their genome necessarily harbors and expresses this (these) cofactor(s), which can in turn be discovered by shRNA depletion.
Objectives: To identify cellular cofactors required for $KRAS^{G12D}$ (but not $BRAF^{V600E}$)-driven NSCLC.

KEYWORDS: Provide a brief list of keywords (limit to 20 words). Kras, Lung cancer, oncogene addiction.

ACCOMPLISHMENTS: The PI is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer whenever there are significant changes in the project or its direction.

- **What were the major goals of the project?**

Specific Aim 1. To identify gene products specifically essential for KRAS-driven NSCLC, we will perform a shRNA screen of thousands of mouse genes, looking for essentiality in multiple independent cell lines derived from two NSCLC GEMMs: one RAF-dependent[1] and one RAS-dependent[2]

Specific Aim 2. To validate our findings in human NSCLC we will test a panel of human NSCLC cell lines with known dependence on RAS[4] for their dependence on the elements (hits) identified in Aim 1.

- **What was accomplished under these goals?**

KRAS mutation is one of the most common oncogenic drivers in lung, pancreatic and colon cancers. Despite having known this for more than three decades, pharmacological inhibition of mutant KRAS in cancers has remained an elusive goal. Our research initiative is driven by the need to discover elements essential for KRAS-driven tumorigenesis. Such elements can then be tested for synthetic lethality-based therapeutic approaches to KRAS-addicted cancers. In order to identify genes specifically required for KRAS-driven cancers, we conducted a pooled short hairpin RNA (shRNA) screen in genetically modified mouse model (GEMM)-derived lung and pancreatic tumor cell lines. These cell lines carried a *KRAS* or *BRAF* mutation. While most of the KRAS mutant lines were KRAS addicted, BRAF mutants were not. Our goal was to find genes that, when depleted, would inhibit the survival of KRAS-addicted tumor cells but not KRAS independent cells. We used a mouse-specific barcoded shRNA library of 27,500 shRNAs targeting 4,625 genes. These genes were selected on the basis of their involvement in cancer-associated signaling pathways and/or drug targeting ability. We briefly describe below how the screen was performed with focus on one of the target resulting from the screen and experiments to test it in lung cancer.

shRNA screen design and analysis: We infected *KRAS*-addicted and *KRAS*-independent mouse cancer cell lines with lentiviruses expressing the shRNA library. Since a lentivirus integrates into the genome of cell, if an shRNA decreases the viability of the cell, its abundance will decrease over the length of the screen. We can observe such "drop out" shRNAs by comparing the relative abundance of shRNAs at the end of the screen to a baseline control representing the library. We infected the cell to get 40% transduction efficiency to ensure that majority of cells were infected with 1 shRNA-carrying virus. The screen was designed to achieve 1000 fold coverage of the library and was carried out to allow 8 to 10 population

doublings post transduction (end point). Abundance of shRNA in the baseline and end point cell is determined by PCR based recovery of barcoded shRNA followed by deep sequencing.

shRNA depletion over time was computed using the R package, Differential expression analysis for sequence count data (DESeq) . Since we are interested in discovering synthetic lethal relation with KRAS, we imposed a "KRAS dropout" cutoff on the screens. KRAS mutant cells that depleted 50% KRAS shRNA (3 out of 6 hairpins) and BRAF mutant cells that did not lose any KRAS hairpins were only selected for the next round of analyses. Potential "hits" are shRNA target genes that drop out in KRAS mutant lines but not in BRAF mutant lines. We prepared 2 separate lists of such genes for lung and pancreatic lines and then looked for hits common to both. This was done to select targets common to different KRAS-driven cancers irrespective of the tissue type. We found 23 hits common to both tumor types.

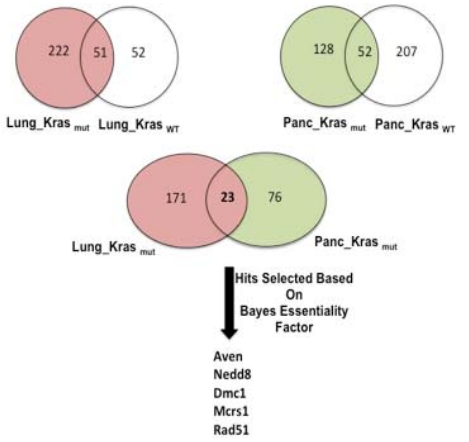

Figure 1: **KRAS-essential Hits** from mouse shRNA screen with the gene *AVEN* at the top of the list.

We further shortlisted the hits by excluding essential genes such as proteasome and polymerase subunits. The short listed genes were then scored using Bayesian classifier of gene essentiality. This gave us 14 potential hits including KRAS (**Figure 1**). Among these, *AVEN*, apoptosis, caspase activation inhibitor gene, showed the strongest correlation to KRAS essentiality. We also confirmed that AVEN was expressed in mouse and human lung as well as pancreatic tumor lines and we selected AVEN for further validation experiments. We validated AVEN essentiality in KRAS-addicted mouse and human lines (**Fig 2A-C**). Importantly, we observed a positive correlation between AVEN and KRAS dependence in human lung and pancreatic lines by AVEN siRNA experiments (**Fig 2D**). In order to further substantiate the relevance of AVEN in lung cancer, we generated an isogenic human lung cell line system to isolate the interaction between KRAS and AVEN. Immortalized human tracheobronchial epithelial cells (AALE) were transformed by expressing oncogenic $KRAS^{G12D}$. Expression of oncogenic $KRAS^{G12D}$ makes the AALE cells KRAS-dependent and confers on them the ability to form colonies in anchorage independent assay. Knocking down AVEN in the KRAS transformed AALE cells significantly reduced their colony formation ability.

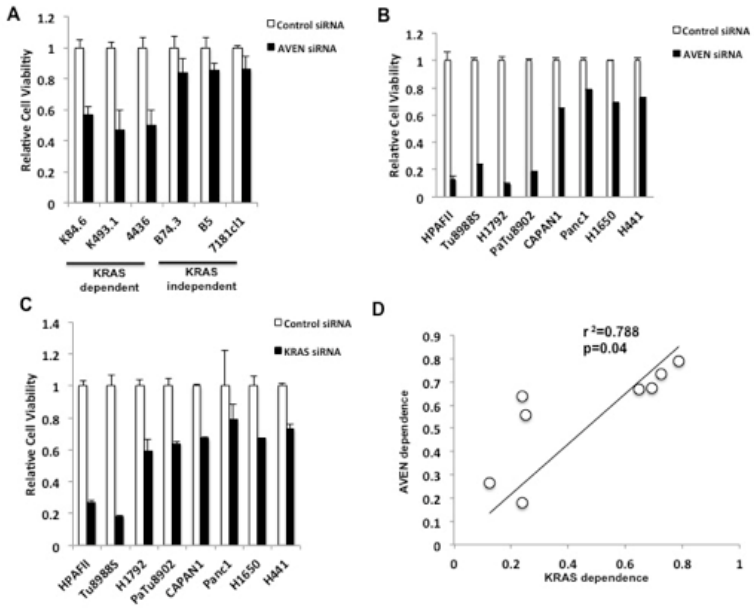

Fig 2: Validation of *AVEN* in mouse (A) and human cell lines (B) Correlation of AVEN and KRAS dependence in human cell lines is plotted (D) using data in panels B and C.

In short, we have convincing evidence that AVEN is a KRAS-essential element in human KRAS-driven cancers. We plan to test this relation *in vivo* as the next step. We hypothesize that AVEN depletion makes KRAS-addicted cells more vulnerable to apoptotic stress. We will test this hypothesis in our future experiments.

What opportunities for training and professional development has the project provided? I require each of the trainees in my lab to complete a written individual development plans (IDP) annually. I meet with each of the trainees in my lab annually to discuss progress during the past year, as well as the development points outlined in their IDP. During the first 2 years of a graduate student's tenure in my lab, I advise the student weekly. For students who have advanced to candidacy, and for postdocs, I advise every

two weeks, and encourage productive goal setting toward the pursuit of a chosen career. I also encourage students and postdocs to attend 4 training seminars provided by the UCSF Office of Career and Professional Development.

- **How were the results disseminated to communities of interest?**
  - "Nothing to Report."
- **What do you plan to do during the next reporting period to accomplish the goals?**
  - "Nothing to Report."

2. **IMPACT:** Describe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to: **This project established a new model system for testing KRAS dependency in cancer. These Findings are being expanded upon. This much larger project is a collaborative effort between many screening scientists looking at various molecular approaches at KRAS inhibition using the cell panel defined herein.**
   - **What was the impact on other disciplines?** This work has set the conceptual underpinnings for the use of mouse derived cancer cell lines for screens in cancers. Future iterations of these screens could, for example, be used with emerging systems such as CAS9-Crispr or small molecule approaches.
   - **What was the impact on technology transfer?** "Nothing to Report."
   - **What was the impact on society beyond science and technology?** "Nothing to Report."
3. **CHANGES/PROBLEMS:** The Project Director/Principal Investigator (PD/PI) is reminded that the recipient organization is required to obtain prior written approval from the awarding agency Grants Officer whenever there are significant changes in the project or its direction. If not previously reported in writing, provide the following additional information or state, "Nothing to Report," if applicable:
   - **Changes in approach and reasons for change**
   - Nothing to Report."
   - **Actual or anticipated problems or delays and actions or plans to resolve them**
   - Nothing to Report."
   - **Changes that had a significant impact on expenditures**
   - Nothing to Report.
   - **Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents**

   Nothing to Report.".
   - **Significant changes in use or care of human subjects**
   - Nothing to Report."
   - **Significant changes in use or care of vertebrate animals.**
   - Nothing to Report."
   - **Significant changes in use of biohazards and/or select agents**

- Nothing to Report."

4. **PRODUCTS:** List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."

   - **Publications, conference papers, and presentations**
     Nothing to Report.

     - **Journal publications.** We are currently validating the AVEN gene using in vivo systems, as proposed in Aim 2. Once these validation experiments are completed, we anticipate submitting this work for publication within the 2015 calendar year.

     - **Books or other non-periodical, one-time publications.** Nothing to Report

     - **Other publications, conference papers, and presentations.** Dr. Malik, the postdoctoral fellow working on this project, has submitted this work for presentation at the 2015 Salk Institute mouse models of human cancer conference.

   - **Website(s) or other Internet site(s)**
     Nothing to Report

   - **Technologies or techniques**
     A new technology of sorts emerged from this grant. That is a collection of mouse cell lines with known KRAS dependence. They will be shared with academic researchers on a collaborative basis upon request.

   - **Inventions, patent applications, and/or licenses**
     Nothing to Report

   - **Other Products**
     Nothing to Report."

   - **PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS**

   - **What individuals have worked on the project?**

     - No Change

   - **Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?**

     - Nothing to Report

   - **What other organizations were involved as partners?**

     - Nothing to Report

5. **SPECIAL REPORTING REQUIREMENTS**

   - **COLLABORATIVE AWARDS:** Not applicable

   - **QUAD CHARTS:** Not applicable

6. **APPENDICES:** N/A